

## Process for the preparation of heterologous bispecific antibodies

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DE4419399 (C1)

EP0763128 (A1)

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Abstract not available for JP 9506001 (T)

Abstract of corresponding document: **DE 4419399 (C1)**

The invention pertains to a method for producing heterologous bispecific antibodies. The object of the invention is a method designed to yield heterologous bispecific antibodies with a high level of purity. This is attained by producing a quadroma fused from hybridomas, one of which produces antibodies that have an affinity to the binding site of protein A and the other of which produces antibodies that have a weaker or no affinity to the binding site of protein A; by propagating and cultivating the quadromas as usual, and by eluting the bsAk in a pH range at least 0.5 units above the pH at which the antibodies with greater affinity to the binding site of protein A are still bonded.

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| C 07 K 1/22              |   | 9356-4H | C 07 K 1/22                |
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| (21)出願番号                 | 特願平8-500228   |         | (71)出願人 ゲーエスエフーフォルシングスツエント |
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| (85)翻訳文提出日               | 平成8年(1996)12月2日   |         | ズントハイト ゲゼルシャフト ミット         |
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| (87)国際公開日                | 平成7年(1995)12月14日  |         | シュライスハイム インゴルシュテッター        |
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| (32)優先日                  | 1994年6月3日   |         | (72)発明者 ホルスト リントホーファー      |
| (33)優先権主張国               | ドイツ (DE)  |         | ドイツ連邦共和国 D-80999 ミュンヘ      |
| (81)指定国                  | E P (AT, BE, CH, DE,<br>DK, ES, FR, GB, GR, IE, IT, LU, M<br>C, NL, PT, SE), CA, JP, US |         | ン トリクスルヴェーク 7              |
|                          |   |         | (72)発明者 シュテファン ティールフェルダー   |
|                          |   |         | ドイツ連邦共和国 D-82229 アイヒェ      |
|                          |   |         | ナウ ビルケンハイム 5               |
|                          |   |         | (74)代理人 弁理士 矢野 敏雄 (外3名)    |

(54)【発明の名称】 異種二重特異性抗体の製造法

## (57)【要約】

本発明は、異種二重特異性抗体の製造法に関する。本発明の課題は、高い純度での異種二重特異性抗体を製造することができるような方法を実施することである。前記課題は、1つのハイブリドーマが、蛋白質Aの結合ドメインに対する親和性を有する抗体を生産し、別のハイブリドーマは、第一の抗体と比べて、蛋白質Aの結合ドメインに対して弱い親和性を有するかまたは親和性を有していない抗体を生産するようなハイブリドーマから融合されたクアドロームの製造、該クアドロームの常法による増殖および培養並びに蛋白質Aの結合ドメインに対してより強い親和性を有する抗体がなお結合しているpHを少なくとも0.5単位上回っているpH範囲内でのbsA kの溶離によって解決される。



























## 文献

[1]

Karawajew L., Micheel B., Behrsing O., Gaestel M.  
. (1987) J. Immunol. Methods 96:265

[2]

Nitta T., Sato K., Yayita H., Okumura K., Ishii  
S. (1990) Lancet 335:368

[3]

MacLean J. A., Su Z., Guo Y., Sy M., Colvin R. B  
. Wong J. T. (1993) J. Immunol. 150:1619-1628

[4]

Brennan M., Davison P. F., Paulus H. (1985) Preparation of bispecific antibodies by chemical recombination of monoclonal immunoglobulin G1-fragments. Science 229:81

[5]

Kostelnik S. A., Cole M. S., Tso J. Y. (1992) J.  
Immunol. 148 : 1547-1553

[6]

Clark M., Gilliland L., Waldmann H. (1988) Hybri

d antibodies for therapy. Monoclonal antibody therapy. Prog Allergy. Basel, Karger, vol45:31-49

[7]

De Lau W. B. M., Heijne K., Neefjes J. J., Oosterweel M., Rozemuller E., Bast B. J. E. G. (1991) Absence of preferential homologous H/L chain association in hybrid-hybridomas. J. Immunol. 146: 906-9 14

[8]

Link B. K., Weiner G. J. (1993) Production and characterization of bispecific IgG capable of inducing T-cell mediated lysis of malignant B cells. Blood 81:3343-3349

[9]

Ey P. L., Prowse S. J., Jenkin C. R. (1978) Isolation of pure IgG1, IgG2a and IgG2b immunoglobulins from mouse serum using protein A-Sepharose. Immunochemistry 15:429-436

[10]

Couderc J., Kazatchkine M. D., Ventura M., Duc H. T., Maillet F., Thobie N., Liacopoulos P. (19

85) Activation of the human classical complement pathway by a mouse monoclonal hybrid IgG1-2a monovalent anti-TNP antibody bound to TNP-conjugated cells. *J. Immunol.* 134:486-491

[11]

Kuppen P. J. K. et al. (1993) The development and purification of a bispecific antibody for lymphokine-activated killer cell targeting against the rat colon carcinoma CC531. *Cancer Immunol. Immunother.* 36:403-408

[12]

Deisenhofer J. (1981) Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from *Staphylococcus aureus* at 2.9 and 2.8 Å resolution. *Biochemistry* 20:2361-2370

[13]

Rousseaux J., Rousseaux-Prevost R. f Bazin H.. Biserre G. (1981) Tryptic cleavage of rat IgG: a comparative study between subclasses. *Immunol. Lett.* 3:93

【図 1】

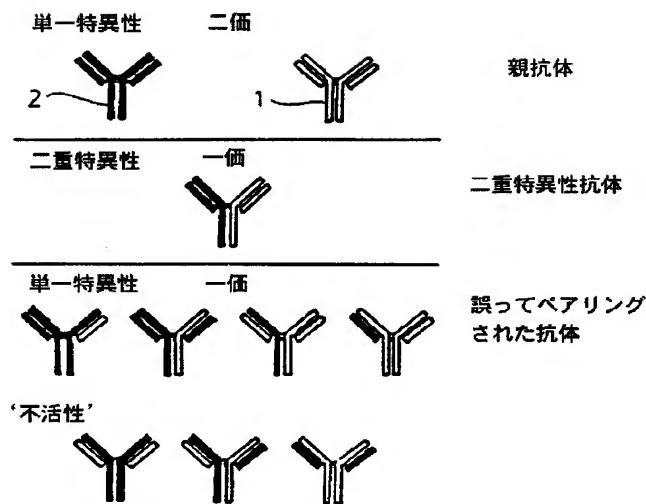
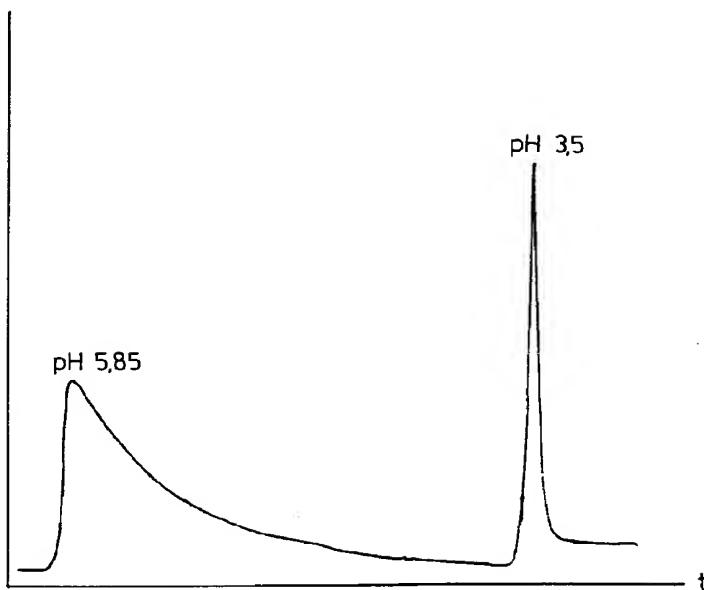


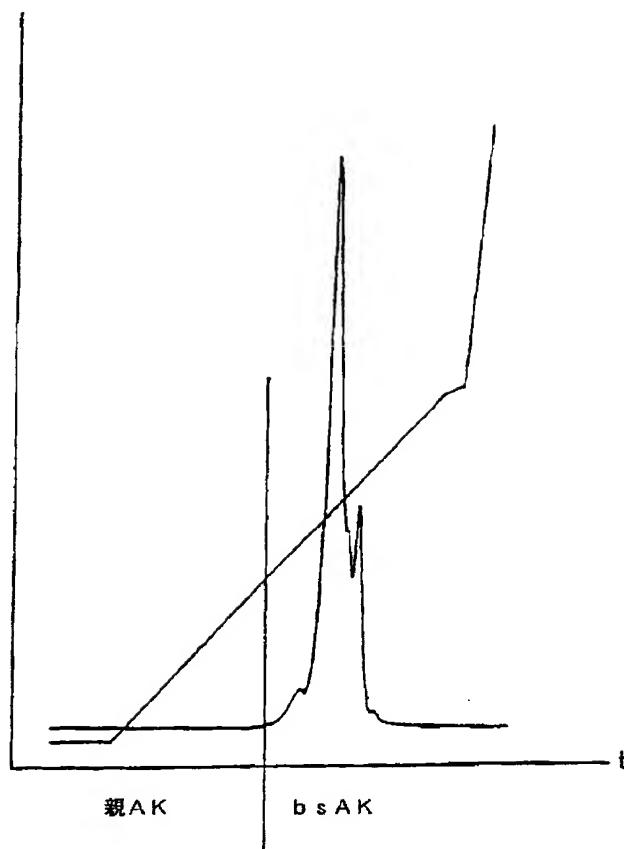
Fig. 2

O.D.280



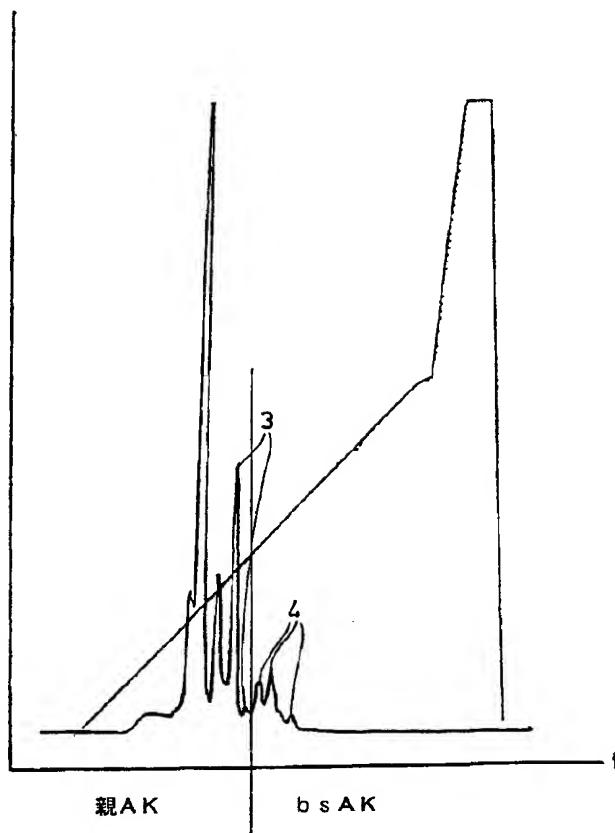
【図 3】

O.D. 280



【図4】

O.D. 280



**INTERNATIONAL SEARCH REPORT**

|  |  |  |
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|  |  | International Application No<br>PCT/EP 95/01850                                |
| CLASSIFICATION OF SUBJECT MATTER<br>IPC 6 C12P21/08 C07K1/22 C07K16/46   |  |  |
| According to International Patent Classification (IPC) or to both national classifications and IPC   |  |  |
| <b>B. FIELDS SEARCHED</b>  |  |  |
| Minimum documentation searched (classification system followed by classification symbols)<br>IPC 6 C12P C07K   |  |  |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  |  |  |
| Electronic data base consulted during the international search (name of data base and, where practical, search terms used)   |  |  |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>  |  |  |
| Category   | Citation of document, with indication, where appropriate, of the relevant passage                                | Relevant to claim No.  |
| X  | GB,A,2 197 323 (NATIONAL RESEARCH DEVELOPMENT CORPORATION) 18 May 1988<br>see examples 2,6<br>see claims<br>---- | 1-4  |
| X  | GB,A,2 197 322 (NATIONAL RESEARCH DEVELOPMENT CORPORATION) 18 May 1988<br>see examples 5,9<br>see claims<br>---- | 1<br>-/-   |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.   |  | <input checked="" type="checkbox"/> Patent family members are listed in annex. |
| * Special categories of cited documents :  |  |  |
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| "Z" document member of the same patent family  |  |  |
| Date of the actual completion of the international search  |  | Date of mailing of the international search report                             |
| 25 September 1995  |  | 13 - 10 - 95   |
| Name and mailing address of the ISA<br>European Patent Office, P.O. 5818 Patentam 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl<br>Fax (+31-70) 340-3016   |  | Authorized officer<br><br>Nooitj, F  |

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Interv. d'Applicatio No  
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| C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |   |                       |
|---|---|-----------------------|
| Category *  | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
| A   | INTERNATIONAL JOURNAL OF CANCER,<br>vol. 55, no. 6, 2 December 1993 GENF, DIE<br>SCHWEIZ,<br>pages 931-937,<br>S. FERRINI ET AL. 'Targeting of T<br>lymphocytes against EGF-receptor+ tumor<br>cells by bispecific monoclonal antibodies:<br>Requirement of CD3 molecule cross-linking<br>for T-cell activation.'<br>see page 932, right column, line 16 - page<br>933, right column, line 8<br>--- | 1-9                   |
| A   | COMPTES RENDUS DE L'ACADEMIE DES SCIENCES,<br>vol. 310, no. 9, 26 April 1990 PARIS,<br>pages 377-382,<br>V. LEBEGUE ET AL. 'Production et<br>caractérisation d'anticorps monoclonaux<br>hybrides présentant une double isotypie<br>IgG1/IgG3.'<br>see "Abridged English version"<br>---   | 1-9                   |
| A   | JOURNAL OF THE NATIONAL CANCER INSTITUTE,<br>vol. 79, no. 6, December 1987 BETHESDA,<br>MD, VSA,<br>pages 1393-1401,<br>M. CLARI ET AL. 'T-cell killing of target<br>cells induced by hybrid antibodies:<br>Comparison of two bispecific monoclonal<br>antibodies.'<br>see the whole document<br>---  | 1-9                   |
| P,O,<br>X   | EXPERIMENTAL HEMATOLOGY,<br>vol. 22, no. 8, 21 August 1994 - 25 August<br>1994 NEW YORK, NY, VSA,<br>page 763<br>H. LINDHOFER ET AL. 'Rat-mouse quadromas<br>allow augmented generation of bispecific<br>antibodies and single step purification.<br>First in vivo studies.'<br>see abstract 321<br>---   | 1-4                   |
| P,X   | IMMUNOBIOLOGY,<br>vol. 191, no. 2-3, September 1994<br>STUTTGART, DEUTSCHLAND,<br>page 248<br>H. LINDHOFER ET AL. 'Preferential<br>species-restricted heavy/light chain<br>pairing in rat/mouse quadromas detected<br>during single step purification of<br>bispecific antibodies on protein A.'<br>see abstract L4<br>-----  | 1-4                   |

## INTERNATIONAL SEARCH REPORT

continued on patent family members

International Application No  
PCT/EP 95/01850

| Patent document cited in search report | Publication date | Patent family member(s) |         | Publication date |
|--|------------------|-------------------------|---------|------------------|
| GB-A-2197323                           | 18-05-88         | AU-B-                   | 616870  | 14-11-91         |
|  |                  | AU-A-                   | 8156887 | 01-06-88         |
|  |                  | EP-A-                   | 0293405 | 07-12-88         |
|  |                  | WO-A-                   | 8803565 | 19-05-88         |
|  |                  | JP-T-                   | 1501201 | 27-04-89         |
| GB-A-2197322                           | 18-05-88         | AU-B-                   | 616871  | 14-11-91         |
|  |                  | AU-A-                   | 8156987 | 01-06-88         |
|  |                  | EP-A-                   | 0289546 | 09-11-88         |
|  |                  | WO-A-                   | 8803565 | 19-05-88         |
|  |                  | JP-T-                   | 1501200 | 27-04-89         |

